Supplementary Material

AFIR derivations for more complex binding scenarios. In developing the AFIR potency metric, a number of assumptions were required. While these assumptions hold true in many clinically relevant scenarios, there are cases where more complex formula for estimating AFIR are required, including:

Dose is not large enough for target to reach its steady state plateau: In this scenario, AFIR will be overestimated by the original equation. This was found to be the case for 5 mg/kg bevacizumab given every two weeks. The calculation of AFIR can be improved by estimating the average steady state target concentration under the dosing regimen of interest to calculate AFIR_{avg}.

Dose is not large enough for drug to be in excess of target: In this scenario, there may not be enough drug molecules to bind every target molecule, such that improving K_{d} no longer improves the potency due to stoichiometric limitations. After a single dose, once the drug concentration drops below the target concentration, it doesn't matter how low K_{d} is, efficacy will be lost, as has been observed using a sensitivity analysis [1]. This scenario occurs for targets at high concentrations, such as C5, where the baseline concentration is 500 nM [2]. This scenario can be accounted for by using the quasi-equilibrium (QE) approximation to calculate AFIR_{QE}.

Drug dissociation rate is very small, making target binding essentially irreversible: In this scenario, most of the drug-target complex is eliminated before the drug can unbind. This can occur for very high affinity drugs. One mechanism for achieving high affinity is when the drug has two different epitopes for the same target molecule, as is the case for hirudin, a protein secreted by leeches to bind thrombin and prevent clotting [3]. An approximation with accounts for irreversible binding (IB) AFIR_{IB}.

Target does not reach steady state plateau. The AFIR and TFIR calculations assume that the total target has reached its steady-state plateau. However, in scenarios where K_{d} is large, even if $D_{\text{tot}} \gg T_{\text{tot}}$, the plateau may not be reached. The closeness of the target to the plateau can be checked by using the Michaelis-Menten Indirect-Response approximation of the TMDD model [4], shown below, ignoring absorption and distribution for simplicity.

$$dD/dt = -k_{\text{eD}}D - \frac{k_{\text{syn}}D}{K_{\text{d}}+D} \quad [1]$$

$$dT_{\text{tot}}/dt = k_{\text{syn}} - k_{\text{eT}}T_{\text{tot}}\left[1-\left(1-\frac{T_0}{T_{\text{tot,ss}}}\right)\frac{D}{K_{\text{d}}+D}\right] \quad [2]$$

For a dose with average drug concentration D_{avg} the average total target at this lower dose ($T_{\text{tot,avg}}$) relative to the plateau level ($T_{\text{tot,ss}}$) is given by:

$$dT_{\text{tot}}/dt = 0 = k_{\text{syn}} - k_{\text{eT}}T_{\text{tot}}\left[1-\left(1-\frac{T_0}{T_{\text{tot,ss}}}\right)\frac{D_{\text{avg}}}{K_{\text{d}}+D_{\text{avg}}}\right]$$

$$\frac{k_{\text{syn}}}{T_{\text{tot,avg}}k_{\text{eT}}} = \frac{T_0}{T_{\text{tot,avg}}} = 1-\left(1-\frac{T_0}{T_{\text{tot,ss}}}\right)\frac{1}{K_{\text{d}}/D_{\text{avg}}+1}$$

$$T_{\text{tot,avg}} = T_0\left[1-\frac{1-T_0/T_{\text{tot,ss}}}{1+K_{\text{d}}/D_{\text{avg}}}\right]^{-1} \quad [3]$$

One can quickly check this result by noting that when $D_{\text{avg}} \to 0$, then $T_{\text{tot,avg}} \to T_0$ and when $D_{\text{avg}} \to \infty$, then $T_{\text{tot,avg}} \to T_{\text{tot,ss}}$. Applying this calculation to omalizumab at 150 mg q2w, bevacizumab at 5 mg/kg q2w, and siltuximab at 3 mg/kg q3w using the parameters in Table 1, gives $T_{\text{tot,avg}}/T_{\text{tot,ss}} = \{0.99, 0.32, 0.91\}$ respectively. Thus for bevacizumab at 5mg/kg q2w, the approximation that target will plateau at $T_{\text{tot,ss}} = k_{\text{eT}}/k_{\text{eDT}}$ is a 3-fold over-estimation, which leads to an overestimation of AFIR and TFIR and an underestimate of target binding. To adjust for this situation, the AFIR and TFIR formulation

can be modified, replacing T_{acc} with $T_{\text{acc-avg}} = T_{\text{tot,avg}}/T_0$ or $T_{\text{acc-min}} = T_{\text{tot,min}}/T_0$, where $T_{\text{tot,min}}$ has the same formulation as $T_{\text{tot,avg}}$ above but $D_{\text{tot,avg}}$ is replaced with $D_{\text{tot,min}}$.

$$\text{AFIR}_{\text{avg}} = K_{\text{d}} \cdot T_{\text{acc-avg}}/D_{\text{tot,avg}} \quad [4]$$

$$\text{TFIR}_{\text{avg}} = K_{\text{d}} \cdot T_{\text{acc-min}}/D_{\text{tot,min}} \quad [5]$$

Drug and target concentration are comparable. Cytokine and growth factor targets are typically present at concentrations that are much lower than the drug concentration, even after target accumulation. However, the drug and target concentrations may be comparable if target turnover is rapid (e.g. CCL2 [5]) or if the target is present at high concentrations (e.g 500 nM for C5 [2], which is targeted by eculizumab [6]). In the calculation of AFIR and TFIR above, it was assumed that the drug was in vast excess to its target and thus $T/T_{\text{tot,ss}} \approx K_{\text{d}}/D_{\text{tot}}$. But when this assumption does not hold, and in particular when there are more target molecules than drug molecules, it does not matter how low K_{d} is, simple stoichiometry demonstrates that it will not be possible to bind all the target. It is this principle that accounts for the limited benefit in reducing K_{d} in extending the duration of the drug effect after a single dose [1].

When this situation arises, there are two possibilities for modeling. If the total target does not approach its plateau, then simulation using the TMDD model is required. On the other hand, if there is minimal target accumulation and the PK remains linear, one can estimate AFIR and TFIR using the Quasi-Equilibrium equations below [7, 8].

$$X = D_{\text{tot}} - T_{\text{tot}} - K_{\text{d}} \quad [6]$$

$$D = \frac{1}{2}\left(X + \sqrt{X^2 + 4 \cdot K_{\text{d}} \cdot D_{\text{tot}}}\right) \quad [7]$$

$$(DT) = \frac{D \cdot T_{\text{tot}}}{K_{\text{d}} + D} \quad [8]$$

$$T = T_{\text{tot}} - K_{\text{d}} \quad [9]$$

Defining the average and maximum target levels ($T^{\text{QE}}_{\text{avg}}, T^{\text{QE}}_{\text{max}}$) based the equation for $T(t)$ above gives us the following estimates for AFIR and TFIR.

$$\text{AFIR}_{\text{QE}} = T^{\text{QE}}_{\text{avg}}/T_0 \quad [10]$$

$$\text{TFIR}_{\text{QE}} = T^{\text{QE}}_{\text{max}}/T_0 \quad [11]$$

In the sensitivity analysis for omalizumab, when lower drug concentrations or higher target concentrations are explored, the accuracy of the AFIR and TFIR theoretical calculation declines due to the large target concentration.

Complex dissociation rate is small. In some scenarios, k_{off} can be very small. For example, hirudin is a protein secreted by leech salivary glands which binds to thrombin to prevent clotting; it has a $K_{\text{d}} = 20$ fM (femptomolar) and achieves this tight binding by simultaneously binding two epitopes of the thrombin molecule [3]; BI1034020 is a drug that binds two different epitopes on Aβ peptide and it could also potentially have a femptomolar K_{d} [9], though to our knowledge, its binding affinity has not been reported. With two binding epitopes, K_{d} can be much lower because even if one bond separates, there is a chance for it to rebind before the other bond disassociates and the drug is free to diffuse away from the target. This scenario, where $k_{\text{off}} \approx 0$, is known as the irreversible binding (IB) approximation and was considered previously [10, 11]. Setting aside absorption and distribution for simplicity, the binding equations with $k_{\text{off}} = 0$ become

$$D' = -k_{\text{on}} D \cdot T - k_{\text{eD}} D$$

If k_{eT} is fast such that the target is in quasi-equilibrium ($dT/dt \approx 0$), this gives

$$\begin{aligned} dT/dt = 0 &= k_{\text{syn}} - k_{\text{on}} D \cdot T - k_{\text{eT}} T \\ 0 &= k_{\text{syn}} - (k_{\text{on}} D - k_{\text{eT}}) T \\ T &= \frac{k_{\text{syn}}}{k_{\text{on}} D + k_{\text{eT}}} \approx \frac{k_{\text{syn}}}{k_{\text{on}} \cdot D} \end{aligned} \quad [12]$$

The AFIR equation for large drug concentration for irreversible binding gives

$$\text{AFIR}_{\text{IB}} = (k_{\text{eT}}/k_{\text{on}})/C_{\text{avg}} \quad [13]$$

$$\text{TFIR}_{\text{IB}} = (k_{\text{eT}}/k_{\text{on}})/C_{\text{min}} \quad [14]$$

In the sensitivity analysis, it was seen that for siltuximab at lower k_{off} rates, the AFIR plateaus, as predicted above. Note that neither k_{off} nor k_{eDT} appear in the AFIR_{IB} and TFIR_{IB} equations. Of course k_{off} is absent because no unbinding occurs and k_{eDT} is also absent because if the drug-complex never disassociates, then the number of complex molecules have no impact on the number of free target molecules.

To understand when this approximation becomes important, consider the quasi-steady-state approximation which assumes that both k_{off} and k_{eDT} are fast and that the drug-target complex (DT) is always in equilibrium. In that case, the equilibrium constant for the system is not K_{d} but rather $K_{\text{ss}} = (k_{\text{off}} + k_{\text{eDT}})/k_{\text{on}}$ [8, 10]. This suggests that when $k_{\text{off}} < k_{\text{eDT}}$, the irreversible binding (IB) approximation may be appropriate.

Complex turnover is slow. The equations for AFIR and TFIR are at steady state. For large doses where $T'_{\text{tot}} \approx k_{\text{syn}} - k_{\text{eDT}} T_{\text{tot}}$, the rate at which the target approaches steady state is k_{eDT} [12] as illustrated by the sensitivity analyses in Figure 3. In Table 1, k_{eDT} ranged from 0.03/d to 0.2. If the time to steady state it taken to be $t_{\text{ss}} = 4/k_{\text{eDT}}$, this gives a time to steady state of 1-4 months and it is after this time that the AFIR and TFIR equations are accurate. If one wants to characterize the target inhibition before steady state, then the full TMDD model should be applied.

Complex turnover is rapid. When the complex turnover is rapid, the quasi-equilibrium (QE) approximation which assumes that $k_{\text{on}} D \cdot T = k_{\text{off}}(DT)$ no longer applies and a better approximation is the quasi-steady-state (QSS) approximation [8], which assumes $(DT)' \approx 0 \rightarrow k_{\text{on}} D \cdot T = (k_{\text{off}} + k_{\text{eDT}})(DT)$. Rapid complex turnover is generally not found in drugs with soluble targets where the complex is cleared at rates comparable to the drug elimination with a half-life of 21 days. But for membrane-bound targets, the complex can be eliminated quickly and this approximation should be considered instead of QE. In this case, the equation for AFIR and TFIR look exactly the same, except that K_{d} is replaced with $K_{\text{ss}} = (k_{\text{off}} + k_{\text{eDT}})/k_{\text{on}}$.

Sensitivity Analyses for Bevacizumab and Omalizumab. Sensitivity analyses for bevacizumab and omalizumab are below. For bevacizumab, AFIR >0.3 and thus the theoretical and numerically calculated values for AFIR do not agree, though the general trends match. For omalizumab, there is generally good agreement between the theory and the simulation, except for low doses and large dosing interval, where the drug concentration and target concentration become comparable.

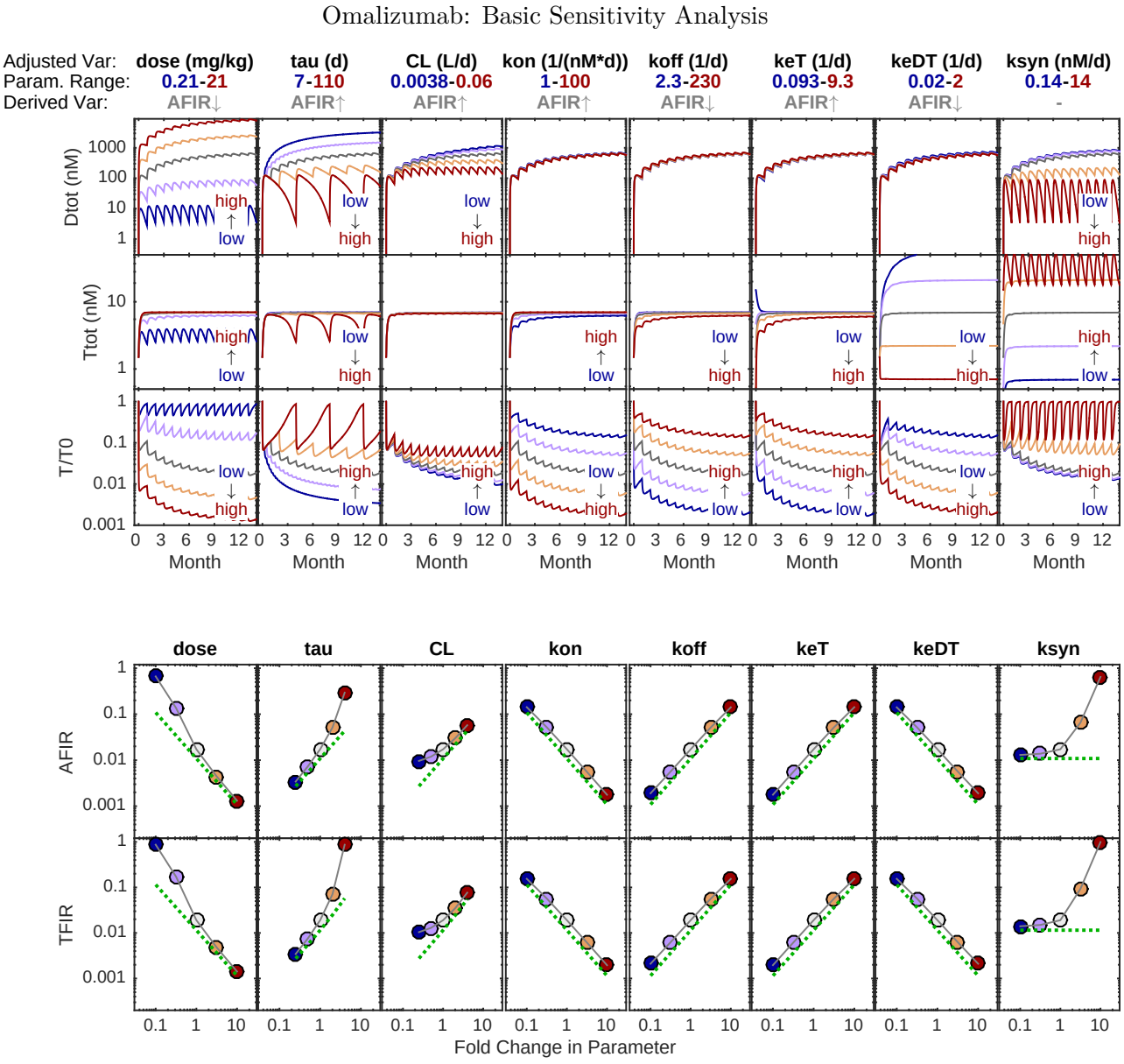

Fig. 1. Basic sensitivity analysis (left) and lumped sensitivity analysis (right) for omalizumab centered about 150 mg dosing every 2 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in Table 1 by either 16-fold (4x lower to 4x higher for CL and τ), or 100-fold (10x lower to 10x higher for all other parameters). Each row represents a different variable of the system. The green dashed line in AFIR and TFIR plots show the theoretical calculation compared to the estimate from the numerical simulation (circles).

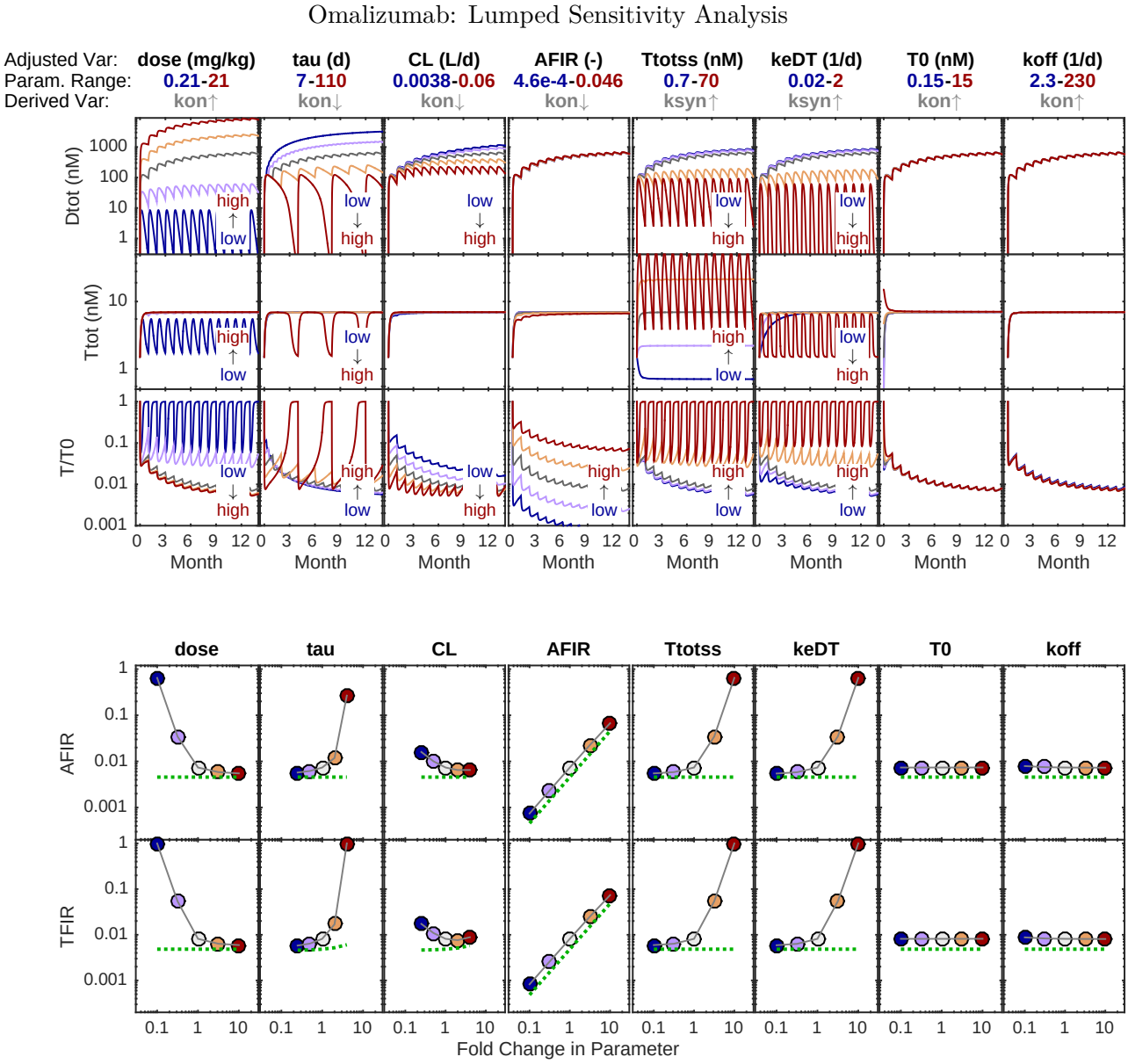

Fig. 2. Lumped sensitivity analysis (left) and lumped sensitivity analysis (right) for omalizumab centered about 150 mg dosing every 2 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in Table 1 by either 16-fold (4x lower to 4x higher for CL and τ), or 100-fold (10x lower to 10x higher for all other parameters). Each row represents a different variable of the system. The green dashed line in AFIR and TFIR plots show the theoretical calculation compared to the estimate from the numerical simulation (circles).

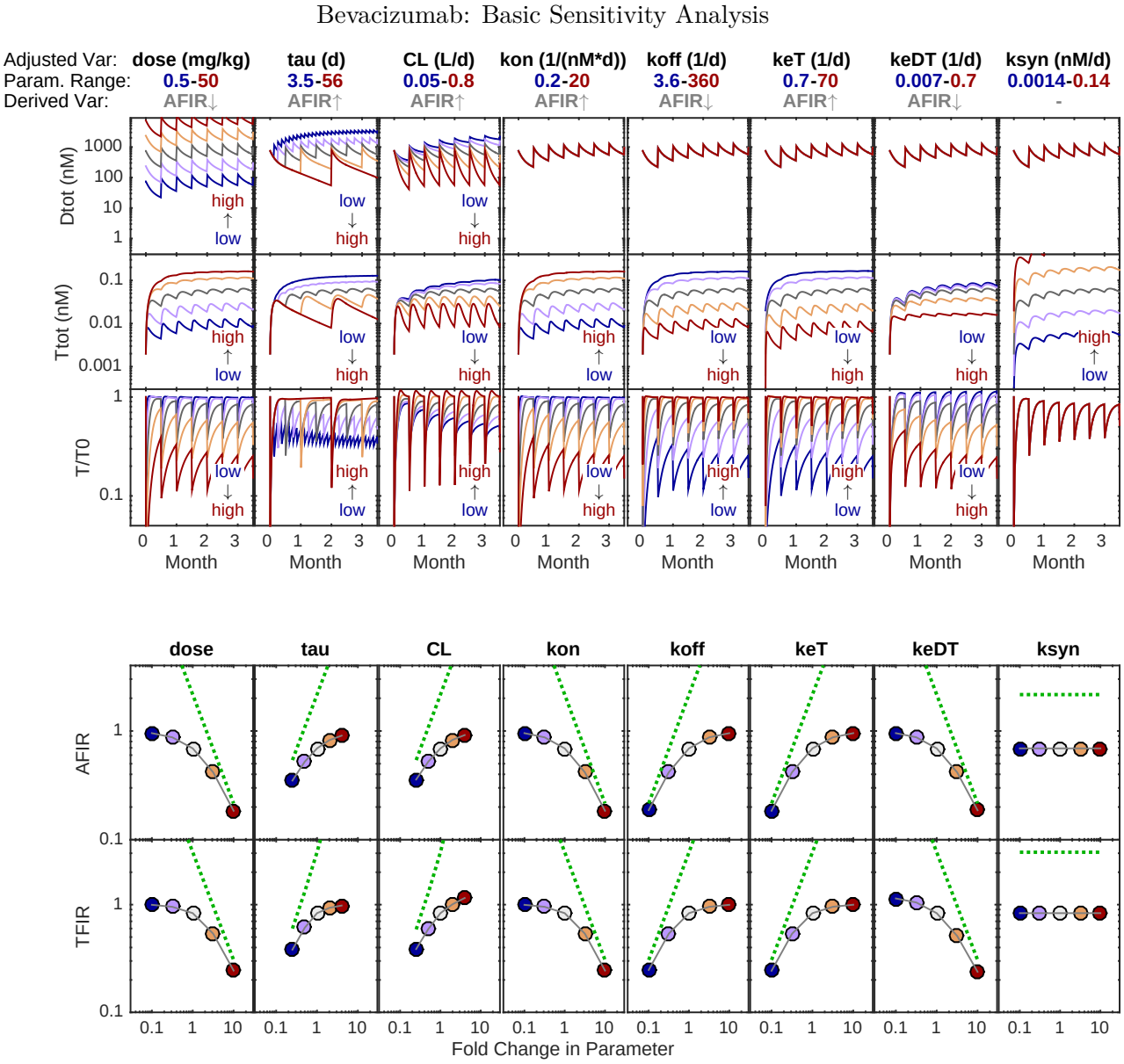

Fig. 3. Basic sensitivity analysis (left) and lumped sensitivity analysis (right) for bevacizumab centered about 5 mg/kg dosing every 2 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in Table 1 by either 16-fold (4x lower to 4x higher for CL and τ), or 100-fold (10x lower to 10x higher for all other parameters). Each row represents a different variable of the system. The green dashed line in AFIR and TFIR plots show the theoretical calculation compared to the estimate from the numerical simulation (circles).

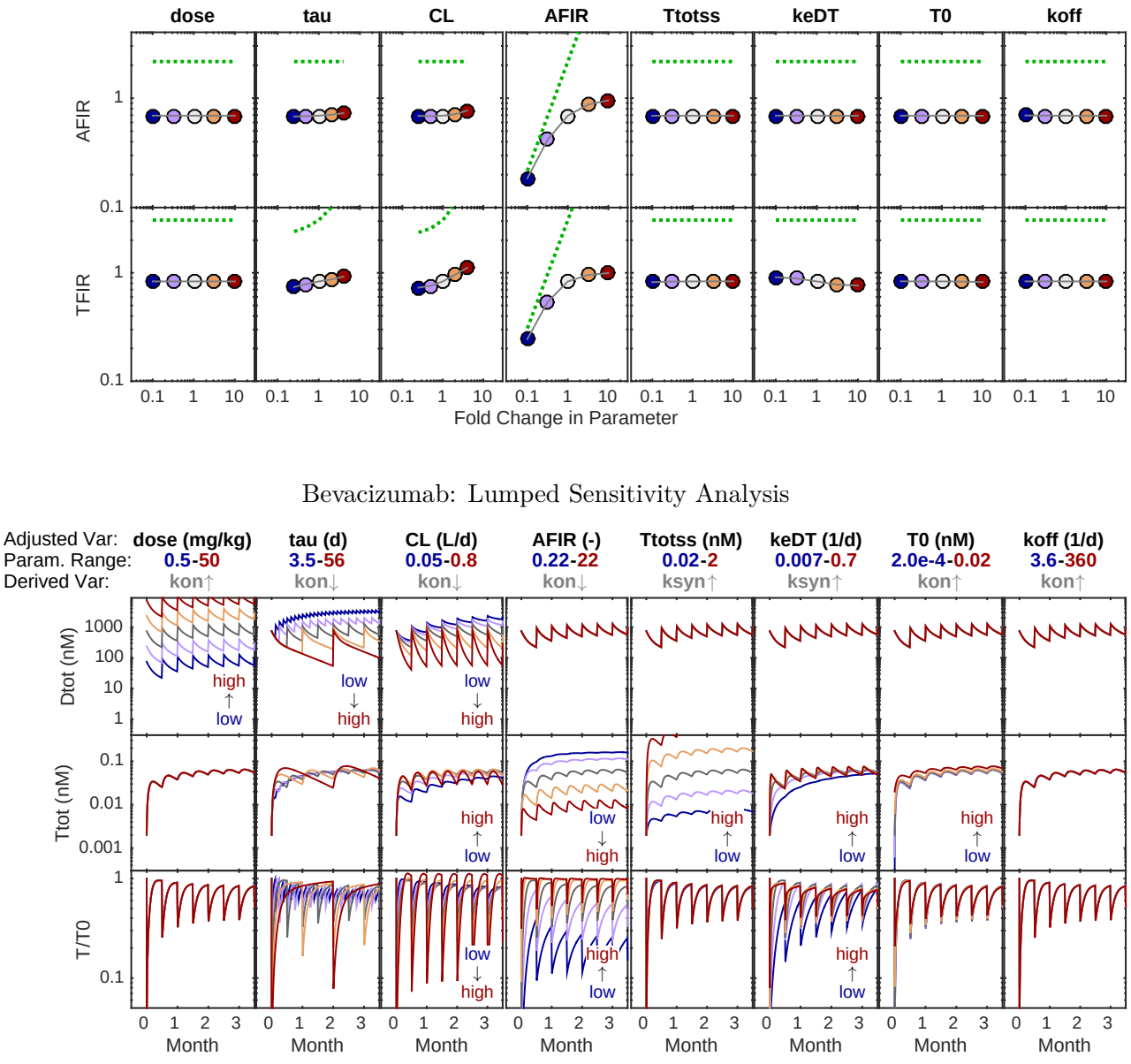

Fig. 4. Lumped sensitivity analysis (left) and lumped sensitivity analysis (right) for bevacizumab centered about 5 mg/kg dosing every 2 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in Table 1 by either 16-fold (4x lower to 4x higher for CL and τ), or 100-fold (10x lower to 10x higher for all other parameters). Each row represents a different variable of the system. The green dashed line in AFIR and TFIR plots show the theoretical calculation compared to the estimate from the numerical simulation (circles).

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